Supplemental Information
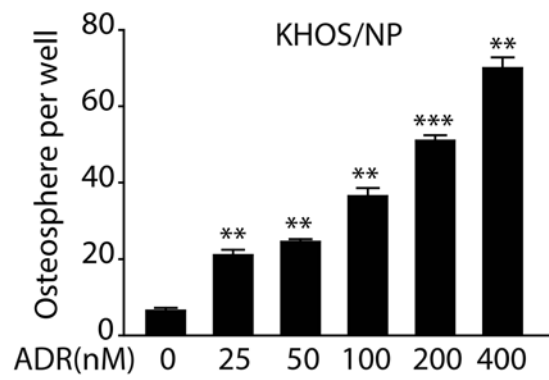
Inhibition of KLF4 by Statins Reverses Adriamycin-Induced Metastasis and Cancer Stemness in Osteosarcoma Cells

Yangling Li, Miao Xian, Bo Yang, Meidan Ying, and Qiaojun He

Supplemental figures and legends

Supplemental Figure S1

Supplemental Figure S1. ADR treatment elevates the sphere formation ability of KHOS/NP cells.
(Related to Figure 1) Cells treated with increasing concentrations of ADR were subjected to the sphere-forming assay. Data presented are the mean $\pm$ SD of three independent experiments. ** $P <$ 0.01, *** $P < 0.001$ *versus* vehicle.
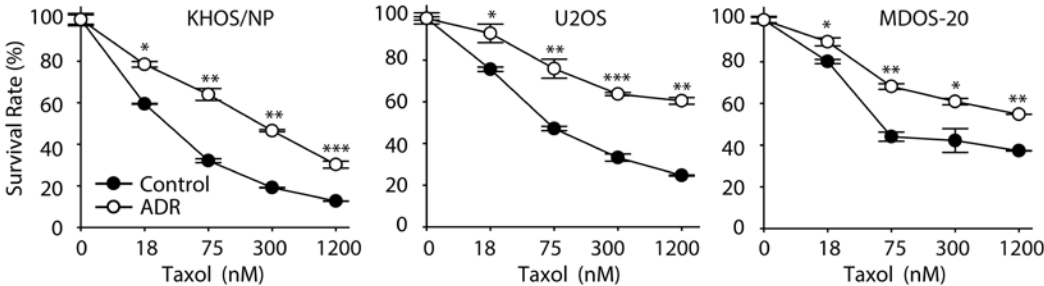
Supplemental Figure S2
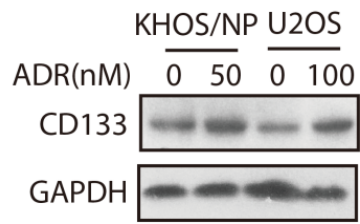
Supplemental Figure S2. ADR treatment increases CD133 protein expression in osteosarcoma cells. (Related to Figure 1) Cell lysates of KHOS/NP and U2OS cells treated with the indicated concentrations of ADR for 24 hr were analyzed by western blotting. Representative images from three independent experiments are shown.

Supplemental Figure S3
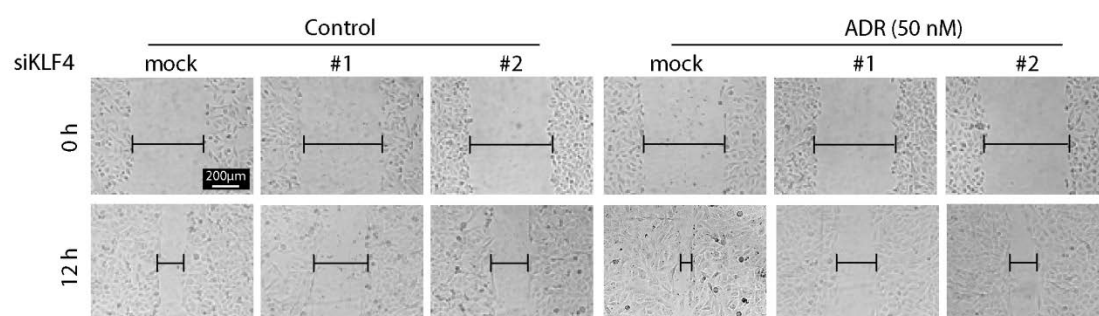
Supplemental Figure S3. KLF4 depletion greatly abolishes would-closure capability of KHOS/NP cells induced by ADR. (Related to Figure 4) KHOS/NP cells were transfected with KLF4 siRNA or Mock (control siRNA) for 48 hr, followed by treatment with ADR (50 nM) for 24 hr, and cell motility was analyzed using the scratch assay. Representative images from three independent experiments are shown.

Supplemental Figure S4

Supplemental Figure S4. ADR treatment results in resistance to taxol. (Related to Figure 1) Osteosarcoma cells were pretreated with vehicle or ADR for 24 hr and then exposed to different concentrations of taxol for 72 hr. Cell viability was determined by the SRB assay. Results represent mean±SD, n=3. * P $\leqslant$ 0.05; ** P $\leqslant$ 0.01; *** P $\leqslant$ 0.001, *versus* vehicle control group.

Supplemental Table S2. Primers used for qRT-PCR amplification

Gene symbol	5'-3'sequence	5'-3'sequence
CD133	GCTTTGCAATCTCCCTGTTG	TTGATCCGGGTTCTTACCTG
ALDH1A1	CACCAGGGCCAGTGTTGTAT	AACACTGTGGGCTGGACAAA
ABCG2	CACCTTATTGGCCTCAGGAA	CCTGCTTGGAAGGCTCTATG
SOX2	TACAGCATGTCCTACTCGCAG	GAGGAAGAGGTAACCACAGGG
KLF4	ACCAGGCACTACCGTAAACACA	GGTCCGACCTGGAAAATGCT
OCT3/4	CTGGAGAAGGAGAAGCTGGA	CAAATTGCTCGAGTTCTTTCTG
NANOG	AAAGAATCTTCACCTATGCC	GAAGGAAGAGGAGAGACAGT
C-MYC	TCCGTCCTCGGATTCTCTGCTCT	GCCTCCAGCAGAAGGTGATCCA
BMI1	AAATGCTGGAGAACTGGAAAG	CTGTGGATGAGGAGACTGC
RAC1	AGACGGAGCTGTAGGTAAAA	GCAGGACTCACAAGGGA
CD24	CAATATTAAATCTGCTGGAGTTTCATG	TCCATATTTCTCAAGCCACATTCA
ID2	CTGTCCTTGCAGGCTTCTGAATTC	CATGAACACCGCTTATTCAGCCAC
DNER	CTCCATTTCTGCATGGGTCT	GAGGAAACCTTGCCAAAACA
MMP2	TGATCTTGACCAGAATACCATCGA	GGCTTGCGAGGGAAGAAGTT
S100P	GATGCCGTGGATAAATTGCT	AGGGCATCATTTGAGTCCTG
SPP1	TCACAGCCATGAAGATATGCTGG	TACAGGGAGTTTCCATGAAGCCAC
CXCR-4	GGTGGTCTATGTTGGCGTCT	TGGAGTGTGACAGCTTGGAG
GAPDH	GTC ATC CAT GAC AAC TTT GG	GAG CTT GAC AAA GTG GTC GT